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Effects of Sex Hormones on the Development of the Platyfish, *Platypoecilus maculatus*[1].

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(Plates I-V; Text-figure 1).

In a discussion of the relation of genic and endocrine factors in sex determination Danforth (1939) used the term *genic* to refer in a general way to the influences emanating from the nucleus and dependent upon its genetic constitution. To the term *environmental* he referred all influences reaching a cell from beyond the limits of its own cytoplasm. He suggested that hormones and organizers even when of autogenous derivation belong to the environmental group. However, he distinguished between the *internal* and *external* environmental agents depending on whether or not they normally arise within the individual.

In an effort to evaluate the part played by the environmental agents determining sex it is desirable to use an organism in which the genetical mechanism for sex determination and the embryological history of the germ cells are known. In the domesticated stocks of the Mexican viviparous platyfish, *Platypoecilus maculatus* Bellamy (1922, 1928), Gordon (1927, 1937), Fraser and Gordon (1929) and others showed that the chromosomal regulation of sex may be expressed by the formula: WZ = female, ZZ = male. The history of the platyfish germ cells from their earliest appearance in the 1.2 millimeter embryos through their post-embryonic development and adult stages were described in great detail by Wolf (1931). He pointed out that definitive ovaries and definitive testes were distinguishable on the day of the platyfish's birth.

The present study is concerned with the effects of the synthetic steroid, pregneninolone upon immature, but genetically determined female platyfish and the effects of the estrogenic substance alpha-estradiol benzoate on immature, but genetically determined male platyfish. Utilizing the genetic sex determining mechanism, we sexed the platyfish within two weeks after birth and applied the hormonal chemicals to the fish at this early age. This paper will describe, but only in a preliminary manner, the effects of the hormones on the developing gonad, skeleton, and sexual behavior. Grobstein (1940, 1942) had previously used the platyfish in studying the effects of testosterone proprionate on the normal and regenerating anal fins of adults. The experimental results reported in the present report are based upon the work of Cohen (1942).

Hormonal regulation of development in poeciliid teleosts, other than *Platypoecilus maculatus*, were studied by Berkowitz (1937, 1938, 1940), Eversole (1939) and Scott (1941, 1944), all of whom used the guppy, *Lebistes reticulatus;* Turner (1941, 1942) used *Gambusia affinis;* and Regnier (1937, 1938), Baldwin and Goldin (1940), Witschi and Crown (1940), Noble and Borne (1940) studied the swordtail *Xiphophorus hellerii.* Much of this and other work was summarized and discussed broadly by Witschi (1939, 1942).

MATERIAL AND METHODS[3]

The genetic method used to sex immature *Platypoecilus maculatus* for these studies was as follows: a black-spotted female of the domesticated stock, heterozygous for the pigmentation pattern, (W)+(Z)*Sp*, was mated to an unspotted, recessive male. (Z)+(Z)+. They produced black-spotted sons (Z)+(Z)*Sp*, and unspotted daughters (W)+(Z)+, see Text-figure 1. The spotted pattern which is made up of clusters of large melanophores was recognized readily within a week of the fish's birth.

Fraser and Gordon (1929) indicated that crossing-over of the sex chromosomes occurred at the rate of about one per cent and this value has been recorded by other geneticists. Thus any error in sex determination, due to genetic factors, could not have been of any consequence. Furthermore, only two instances of complete and functional sex reversals have been reported during 24 years of genetic work with these fish (Gordon, 1946).

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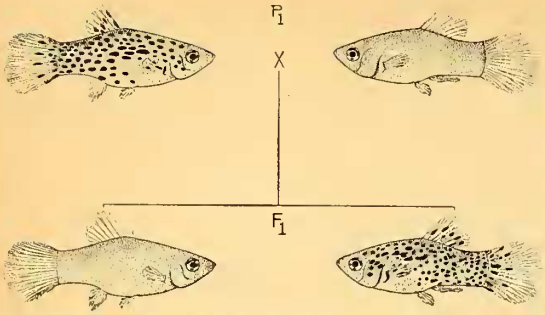

TEXT-FIG. 1. The genetic method that was used to sex immature domesticated *Platypoecilus maculatus*. A black-spotted female of the domesticated stock, heterozygous for the spotted pattern, was mated to an unspotted, recessive male. These are shown on the top line. They produced black-spotted sons and unspotted daughters, as shown on the second line. The spotted pattern may be recognized on the day of birth of the fish or within a week thereafter. (After Gordon, 1932).

Fifteen immature but genetically determined males were treated with one milligram of crystalline alpha-estradiol benzoate once a week; nineteen immature but genetically determined females were treated with five milligrams of the synthetic steroid pregneninolone once a week. Thirty-one fish were used as controls. The fish were kept in water, temperature at approximately 25°C., in three gallon glass aquaria, six animals to each tank. The hormonal substances were given to the fish beginning at an age of two weeks. Some of the animals were treated for eight weeks while others were given the hormones for twelve weeks, and still others received treatment for a maximum of twenty weeks. The fish swallowed many of the crystals as they fell through the water. Some of the crystals might have dissolved partially in the water and affected the fish directly.

All the fish were fed similarly with commercial fishfoods, dried shrimp, dried liver and occasionally they were given live tubifex worms. At the conclusion of the experiments the animals were fixed in Bouin's picro-formol fluid; the fish were measured, dissected, their gonads removed, sectioned and prepared for microscopical examination by staining with Harris' hematoxylin alum and eosin. When gonopodia were induced, these organs were removed and mounted for study in balsam or glycerin.

EXPERIMENTAL RESULTS

1. *Effect of alpha-estradiol benzoate on gonads of immature, genetic male platyfish.*

In the untreated, control males, the testes were found to be *fused* and relatively large. The sperm duct epithelial cells were cuboidal, the spermatophores were abundant and all the other stages in spermatogenesis were found. In the estrogen-treated males, the gonads were small, compact and appeared bipartite, the two lobes being separated by a membrane. The cells of the sperm duct epithelium were columnar; the interstitial tissue was profuse; a number of large ova were seen but spermatophores and spermatids were absent. The testicular elements that remained were spermatogonia. The general picture seen was one of a radical modification of development of the testes. As the treatment was continued the effects instead of continuing along the lines of greater modification, changed slightly in the direction of the controls. Thus after 20 weeks of treatment, the gonads were found to contain some spermatophores and none of them contained ova.

2. *Effect of pregneninolone on gonads of immature, genetic female platyfish.*

In the untreated, control females, the ovaries were fairly large, containing many oocytes and ova; the latter had abundant yolk material. In the pregneninolone-treated females, the ovaries were markedly changed; they were small and contained only a few oocytes. Mature ova with their usual complement of yolk were entirely wanting. The modified ovaries appeared definitely degenerate. With continuing treatment, up to 20 weeks, the conditions of gonadal degeneration were maintained. Within a single modified ovary a structure resembling a sperm duct was discovered (Fig. 8).

3. *Effect of sex hormones on certain skeletal elements.*

(*a*) *Gonopodium, the modified anal fin.*

In genetic males in which the testes had been modified by estrogenic substance to a point where no mature sperms or secondary spermatocytes were present, the anal fins were not transformed. However, in those fish where the treatment with estrogens did not completely inhibit spermatogenesis and mature sperm were present, perfect gonopodia were found.

Genetic females treated with pregneninolone for only two weeks developed gonopodia which, however, were aberrant in form. Similar abnormalities were described by Grobstein (1942) when he treated adult females with testosterone after removing their anal fins.

(*b*) *Gonopodial Suspensorium.*

Pregneninolone-treated females in which gonopodia were developed moved these fins just as normal males moved their gonopodia. Histological preparations of the internal skeletons of the modified females revealed that they had developed typical, male-like gonopodial suspensoria. The gonapophyses were well developed and the connections between these and the gonactinosts were present. The gonopodial muscle was strongly developed. The induced skeletal elements conformed to the descriptions of the species by Langer (1913) and by Gordon and Benzer (1945). Turner (1942) reported

TABLE I.

EFFECT OF ALPHA-ESTRADIOL BENZOATE ON GONADS OF DEVELOPING MALE PLATYFISH.

	Estrogen	*Control*	*Estrogen*	*Control*	*Estrogen*	*Control*
No.	4	4	5	5	6	5
Age	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
Duration	8 Weeks	8 Weeks	12 Weeks	12 Weeks	20 Weeks	20 Weeks
Gross Gonad	Small, bipartite, compact	Loose, fused	Small, bipartite, compact (fig. 3)	Large, loose, fused (fig. 1)	Small, bipartite, compact	Large, loose, fused
Sperm Duct Epithelium	Columnar	Low, cuboidal	Columnar	Low cuboidal almost squamous	Columnar	Low cuboidal
Interstitial Tissue	Profuse	Scant	Profuse (fig. 4)	Scant (fig. 2)	Profuse	Scant
Presence of Ova	Large ova (fig. 5)	Absent	Absent	Absent	Absent	Absent
Spermatophores	Absent	Profuse	Absent	Profuse (fig. 1)	Few	Profuse
Spermatids	Absent	Present	Absent	Present	Few	Few
Physiological Status	Inhibited	Active	Inhibited	Highly active	Slightly active	Highly active

similar results in treating female *Gambusia*, while Scott (1944) obtained them in *Lebistes*.

(*c*) *Caudal fin rays.*

In females treated with pregneninolone the shortening of the 7th, 8th and 9th caudal fin rays, and the lengthening of the 6th fin ray were induced. These changes produced a tiny sword-like extension on the tail fin, a feature which is characteristic of a related species, *Platypoecilus xiphidium*. In normal *P. maculatus* the caudal fins were symmetrical; in addition, the fin rays are strong and their terminal elements bifurcate at least once. The fin rays of treated females were not bifurcate at their tips and they were of a smaller dimension than those of the normal animals (Figs. 10, 11, 12).

4. *Effect of sex hormones on total body size and form.*

The females treated with androgens approached the body form and size of normal males (Table III). The males treated with estrogens resembled normal females. The values for body form were determined by dividing the value of their body length (not including tail fin; this is the standard length) by the value of their body width (measured from the base of anterior margin of the dorsal fin to the mid-point between the origins of the pelvic fins).

Normal female platyfish exceeded the males in standard length. The treated females showed the generalized effects of the androgens by their relatively smaller size. On the other hand, the estrogen-treated males attained a size greater than did their controls. These results confirmed those obtained by Scott (1944) in *Lebistes*.

5. *Effects of sex hormones on sexual behavior.*

Masculinized females when placed in an aquarium with normal females were aggressive, pursued the normal females, thrust their gonopodia toward them and attempted to copulate with them.

Males feminized by treatment with estrogens for seven weeks when placed in an aquarium with normal females made no attempt to pursue or mate with them. Later when the normal females were removed and were replaced with normal males, the normal males pursued the feminized males and attempted to mate with them. These observations were carried out two or three times a week for a period of a month.

TABLE II.

EFFECTS OF PREGNENINOLONE ON GONADS OF DEVELOPING FEMALE PLATYFISH.

	No. of ♀	*Duration of Experiment*	*Size of Gonad*	*Oocytes*	*Ova*	*Yolk Deposition*	*Physiological Status*
Treated	19	5-20 Weeks	Very small (fig. 7)	Very few present	Absent	Inhibited	Inhibited (fig. 9)
Control	19	5-20 Weeks	Large (fig. 6)	Many present	Present	Active	Active

TABLE III.

FORM INDEX[1].

Alpha-estradiol Benzoate-treated Males				Pregneninolone-treated Females		
Length Cm.	*Width Cm.*	*Form Index*		*Length Cm.*	*Width Cm.*	*Form Index*
1.95	.65	3.0		1.55	.45	3.4
2.2	.70	3.14		1.9	.50	3.8
2.4	.85	2.8		2.05	.65	3.18
2.35	.80	2.9		1.3	.40	3.25
2.3	.70	3.2		1.9	.50	3.80
1.9	.70	2.7		1.4	.40	3.50
1.7	.60	2.8		1.4	.40	3.50
2.2	.80	2.75		1.55	.45	3.40
1.75	.60	2.9		1.50	.45	3.33
1.85	.70	2.6		1.40	.40	3.50
Mean		2.90	$t = 6.7$ $P < 0.001$	Mean		3.50
Normal Males				Normal Females		
1.75	.50	3.50		2.1	.74	2.8
2.20	.60	3.67		2.1	.75	2.8
1.73	.45	3.80		2.1	.75	2.8
1.75	.50	3.50		1.8	.70	2.56
1.75	.50	3.50		2.55	.85	3.00
1.73	.45	3.80		2.6	.90	2.90
1.75	.45	3.50		2.6	.90	2.90
Mean		3.61	$t = 9.8$ $P < 0.001$	Mean		2.82

[1] The data on form index differences in males and females were treated according to the methods described by Simpson and Roe in Quantitative Zoology (1939:210-211) for the comparison of the means of two small samples. The data are significant.

DISCUSSION

The newly born platyfish is about 6.5 mm. long. At this stage, according to Wolf (1931) who traced the history of their germ cells, sex differentiation has already occurred. In the immature female, the paired gonads are fused medially; the germ cells have multiplied, spread throughout the body of the gland and enlarged to two or three times their original size. A number of stroma cells surround them, forming oocytes, and this is the first indication of follicle formation. When pregneninolone is applied to two-week-old fishes, apparently oogenesis is inhibited beyond oocyte development for no ova were found after 5, 8, 11 and 20 weeks of treatment; in one female a sperm duct-like structure was found. The gonads of the control fish contained all stages of oogenesis including yolk-filled ripe ova. These results were similar, for the most part, to work on female swordtails and guppies.

The work of Vivian (1939) and Matthews (1939), who hypophsectomized *Fundulus* females and found that maturation of the primary oocytes was suppressed, and the work of Hasler, Meyer and Field (1939) on inducing trout to spawn prematurely with the aid of pituitary glands of the carp, are suggestive of the possibility that the pituitary function in female platyfish was inhibited by the application of pregneninolone. This appears likely for not only were the oogenesis processes interrupted in treated platyfish, but the larger size of the normal female was never reached by pregneninolene-treated females.

In the early postnatal male platyfish Wolf found that the germ cells are pushed out to the periphery of the gland, so that the center of the embryonic testis is occupied by stroma cells only, and this condition he reported is similar to the one found in *Xiphophorus hellerii*. The stroma cells cluster to form a duct which is destined to be the sperm duct. In slightly older fish (9 to 12 mm.) Wolf found that the gonads are united and partially fused, but even the mature testis shows its bilateral origin in its bilobed contour, but it is not bipartite. In the normal 16 mm. or late juvenile testis, spermatocytes only are present; indeed, at this stage there seems to be a great deal of degeneration of the sex cells in the normal fish. This degeneration is attributed to the rapid proliferation of the germ cells with an inadequate blood supply for all of them. Wolf is convinced, however, that this degeneration of some germ cells has no significance in the history of the germ cells. In the definitive adult gonad, in *Platypoecilus* and in *Xiphophorus*, Wolf found no seminiferous tubules. Acini form from preexisting acini at the periphery of the testis, which in turn are descended from the primordial germ cells, and as they form they are

pushed in toward the center of the testis. Wolf is emphatic in his belief that neither the peritoneal covering of the testis, the stroma cells, nor the cells of the sperm duct transform into sex cells.

The generalized effects of treating genetically determined male platyfish with alpha-estradiol benzoate was the suppression of spermatogenesis beyond the spermatocyte stages and in the induction of ovo-testes in some instances. In males treated for 8 weeks large ova were found; in males treated for 12 weeks a few small oocytes were found, while in males treated for 20 weeks no oocytes or ova were seen. This indicates a lessening of effectiveness of the estrogen. In the early stages of the treatment an insufficient testicular secretion may have been overruled by the applied estrogen; in more mature males the normal testicular secretion may have been sufficient. The generalized inhibitory effects of normal testicular development by estrogens have been reported in the swordtail and guppy. However, the experiments of Crown (1941) described briefly by Witschi (1942) show that many *Xiphophorus* females may become masculinized by estrone, too. Their ovaries regress, the anal fin transforms incompletely into a gonopodium and the sword characteristic of the male tail fin begins to form. One might wish that the genetic sex determining mechanism in the swordtail was as clear as that in the platyfish. Nevertheless, we have one confirmatory fact to add in this connection. We had a gravid female platyfish which produced a brood under normal conditions. It was then subjected to alpha-estradiol benzoate treatment for three months; at frequent intervals it was allowed the company of a fertile male platy under normal conditions. After the three month period, the treatment was discontinued. While under the influence of the estrogen, it did not produce any further broods, but after the fourth month, six weeks after the treatment was over, it had another brood. In this instance the estrogenic hormones may have affected the sperm adversely within the oviduct, or they may have prevented oogenesis.

Gardner and Pfieffer (1945) reviewed the subject of the influences of estrogens and androgens on the skeletal systems of higher vertebrates, but gave only passing attention to fishes. Grobstein (1941) found that gonopodia regenerated in place of normal anal fins in female platyfish when subjected to testosterone treatment, and similar results were found in *Gambusia* by Turner (1941, 1944) and in *Mollienisia* by Cummings (1943).

The gonopodium articulates with a complex internal skeletal and muscular mechanism which makes possible the movement of the copulatory fin. The hemal spines of the first three caudal vertebrae become modified into gonapophyses and the anterior interhemal spines become modified into a series of fused gonactinosts; in addition, a powerful gonopodial muscle is developed in normal adult male platyfish. The skeletal gonopodial suspensorial elements in the male platyfish were first described by Langer (1913) and more recently in a comparative way by Gordon and Benzer (1945). Similar gonopodial suspensorial elements were induced in treating young females with pregneninolone. This androgen also modified the structures of the caudal fin, the most interesting effect being the induction of a tiny sword in female *Platypoecilus maculatus* which is taxonomically diagnostic of *Platypoecilus xiphidium*. This feature was discussed previously by Gordon, Cohen and Nigrelli (1943). In addition, we have found a specific effect of androgens in suppressing the tertiary bifurcation of the caudal fin rays and in reducing the calibre of the rays.

Berkowitz (1938) showed that estrogens increased the size of *Lebistes* males beyond that usually attained by them, and Eversole (1941) found that androgens reduce the size attained by female guppies. In our experiment, these general results were confirmed in the platyfish. In addition, the body contour characteristic of the normal female was induced in estrogen-treated males and that of the male was obtained in pregneninolone-treated females. Gerschler (1914) and Bellamy (1922) measured platyfish and found that the greatest depth divided into the standard length produced the body index value of 2.7 in both sexes, but Chambers (unpublished) found that females had a greater depth than the males, and our figures are in line with those of Chambers (1935).

Noble and Borne (1940) showed that androgens would elevate the rank of an individual swordtail in the social hierarchy of a group living under aquarium conditions. Androgen treatment caused female guppies to act like males in their great sexual drive, according to Eversole (1940, 1941). The difference in the sexual behavior in *Lebistes* is great; the females (according to Breder and Coates, 1935) show no response whatever, while the males are ceaseless in their courtship activities.

Braddock (1945) found that a social hierarchy is present in a group of platyfish. We found that the behavior patterns in platyfish may be reversed completely by the treatment of females with pregneninolone and the treatment of males with alpha-estradiol benzoate, and this is in line with the results of others working with many species of fishes and other vertebrates.

In summarizing the work on hormonal regulation of development in lower vertebrates, Witschi (1942) claimed that sex hormones

have relatively little to do with primary sex differentiation, and that the inductor substances, which play their roles at earlier stages of the ontogenetic process than sex hormones, belong to a separate class of chemical materials. It appears then in order to tackle this general problem of the influence and nature of the internal and external environmental agents in sex-determination, it is desirable to know accurately and in advance the genetic sex of an individual before treatment. For this purpose the platyfish, *Platypoecilus maculatus*, and their sex-linked characters might provide the suitable test animals.

Summary.

Alpha-estradiol benzoate suppressed spermatogenesis but stimulated the development of connective and interstitial tissues in genetic, immature male *Platypoecilus maculatus*. The transformation of the anal fins into gonopodia was prevented. Young males treated for 8 to 12 weeks showed ova in ovo-testes; males treated 20 weeks showed no ova and indicated a falling off of the inhibiting effects of the estrogen.

Pregneninolone suppressed oogenesis and yolk formation in genetic, immature female platyfish. The effects did not diminish in animals treated for 20 weeks; on the contrary, the ovaries showed greater degeneration. The following male-like characters appeared: gonopodia, elements of the gonopodial suspensorium: gonapophyses and gonactinosts. In addition, tiny sword-like extensions appeared in the caudal fins. Bifurcations of the fin rays did not proceed beyond the primary stage.

Immature females treated with pregneninolone developed a body index and body size characteristic of normal males, while immature males treated with alpha-estradiol benzoate developed the body index and body size characteristic of normal females.

Pregneninolone-treated females developed courtship behavior patterns characteristic of males; they became aggressive; pursued and thrust their gonopodia toward and attempted to copulate with normal mature females. Estrogen-treated males behaved like females; they lost their aggressiveness and normal males pursued them. None of the sexually reversed males or females were fertile.

The use of genetic methods for the determination of the genic sex of the immature animal was suggested as a desirable tool in evaluating the effects of the environmental agents like sex hormones on sexual development.

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EXPLANATION OF THE PLATES

Plate I.

Fig. 1. *Testis of Normal Male*, 12 *Weeks Old.* This normal testis is fused with no evidence of an earlier developmental bipartite gonad. All stages of spermatogenesis including many spermatophores (*Sp*) are represented. The interstitial tissue is scant; the gonad as a whole is loosely constructed. 100×.

Fig. 2. *Testis of Normal Male*, 12 *Weeks Old.* This is same as above but under greater magnification. The deeply staining ring-like structures are spermatophores (*Sp*) containing mature sperms, ripe and ready for ejaculation. The spermatids and other stages of spermatogenesis (*Sr*) are represented by the lighter-staining roundish masses. 440×.

Fig. 3. *Testis of Estrogen-treated Male*, 12 *Weeks Old.* This testis of a treated male shows the bipartite structure (*P* to *P*). Spermatogenesis has only proceeded to the stage of formation of spermatogonia and spermatocytes (*Sr*). Spermatids, mature sperms and spermatophores are wanting. 100×.

Plate II.

Fig. 4. *Testis of Estrogen-treated Male*, 12 *Weeks Old.* This is the same as Fig. 3 but under greater magnification. The testis is compact with a greater amount of interstitial tissue (*N*) than is found in an untreated male. Spermatogonia and spermatocytes (*Sr*) are the prevailing germinal elements. 440×.

Fig. 5. *Ovo-testis of Estrogen-treated Male*, 8 *Weeks Old.* An oocyte (*O*) is present in the upper left portion of the figure. The interstitial tissue is profuse throughout the gonad. 440×.

Plate III.

Fig. 6. *Ovary of Normal Female*, 8 *Weeks Old.* The ovary is large and occupies the greater portion of the abdominal cavity. The ova (*ov*) are well developed and well filled with yolk (*Y*). The smaller circular masses are oocytes (*o*). In sectioning the ovary, the yolk within the ova fragments. 100×.

Fig. 7. *Ovary of Pregneninolone-treated Female*, 8 *Weeks Old.* The ovary is very small and compact; compare this figure with Fig. 6 which is reproduced at the same magnification. The dominant germinal elements present are oocytes (*o*). No yolk is present. 100×.

Fig. 8. *Ovary of Pregneninolone-treated Female*, 8 *Weeks Old.* This ovary is shown under higher magnification. It is a tubular structure which resembles a testicular sperm duct (*Sd*). Oocytes (*o*) are found throughout. 100×.

Fig. 9. *Abdominal Region of a Pregneninolone-treated Female*, 11 *Weeks Old.* The ovary is represented by a few scattered oocytes (*o*) and these germinal elements do not contain yolk. Portions of the intestine (*i*) are shown. 100×.

Plate IV.

Fig. 10. *Caudal Fin Rays of Normal Female.* The fin rays near their origin have a greater diameter than those shown below. At their terminal points shown here, the fin rays show a secondary (2) bifurcation. The primary (1) bifurcation may be seen through the center of the figure. The dots represent the micromelanophores. 5×.

Fig. 11. *Caudal Fin Rays of a Pregneninolone-treated Female.* The fin rays have a narrower diameter than those of the normal shown above. The rays show only a primary (1) bifurcation. Owing to the shortening of the 7th, 8th and 9th fin rays and a lengthening of the 6th, a tiny sword-like strutcure was induced (*S*), a feature which resembles the "sword" of a related species, *Platypoecilus xiphidium.* See Fig. 12. 5×.

Plate V.

Fig. 12. *The Platyfishes: Platypoecilus maculatus and xiphidium.* The male *P. maculatus* is on the left and the female is on the lower right. *P. xiphidium* male is on the upper right. Note the short "sword" in *P. xiphidium* male. The fin rays of all the fishes have primary and secondary bifurcations. The male *P. maculatus* has no "sword". In this paper it has been shown that a "sword" may be induced in female *P. maculatus* by the application of the androgenic substance, pregneninolone.

FIG. 1.

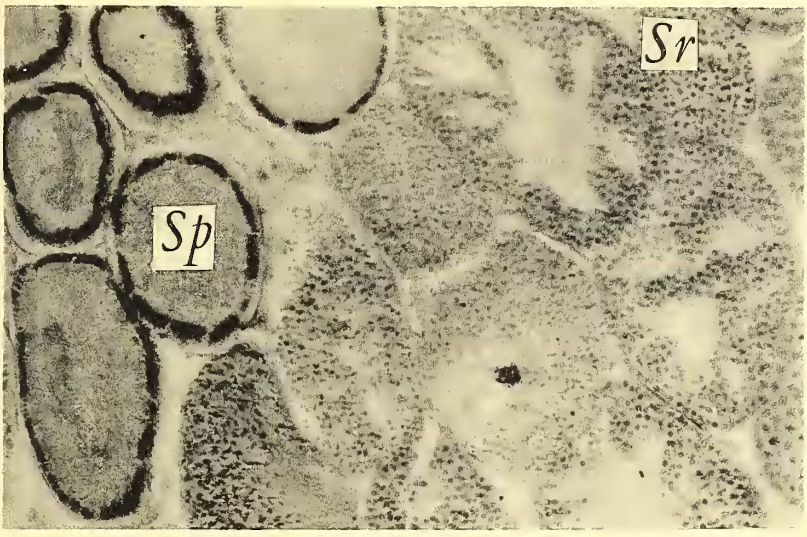

FIG. 2.

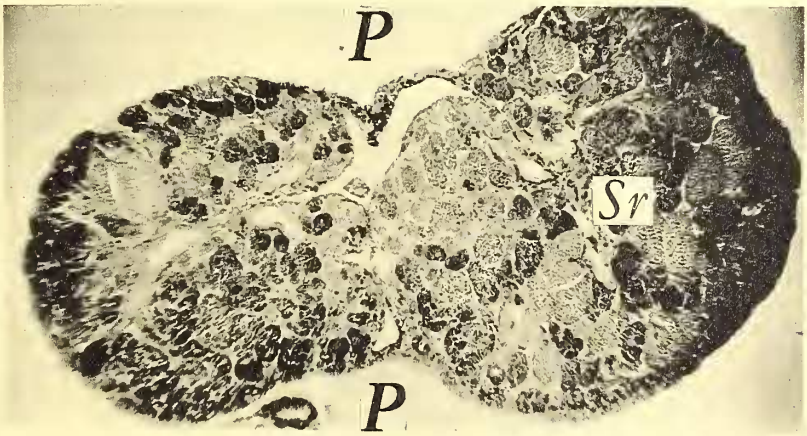

FIG. 3

EFFECTS OF SEX HORMONES ON THE DEVELOPMENT OF THE PLATYFISH, PLATYPOECILUS MACULATUS.

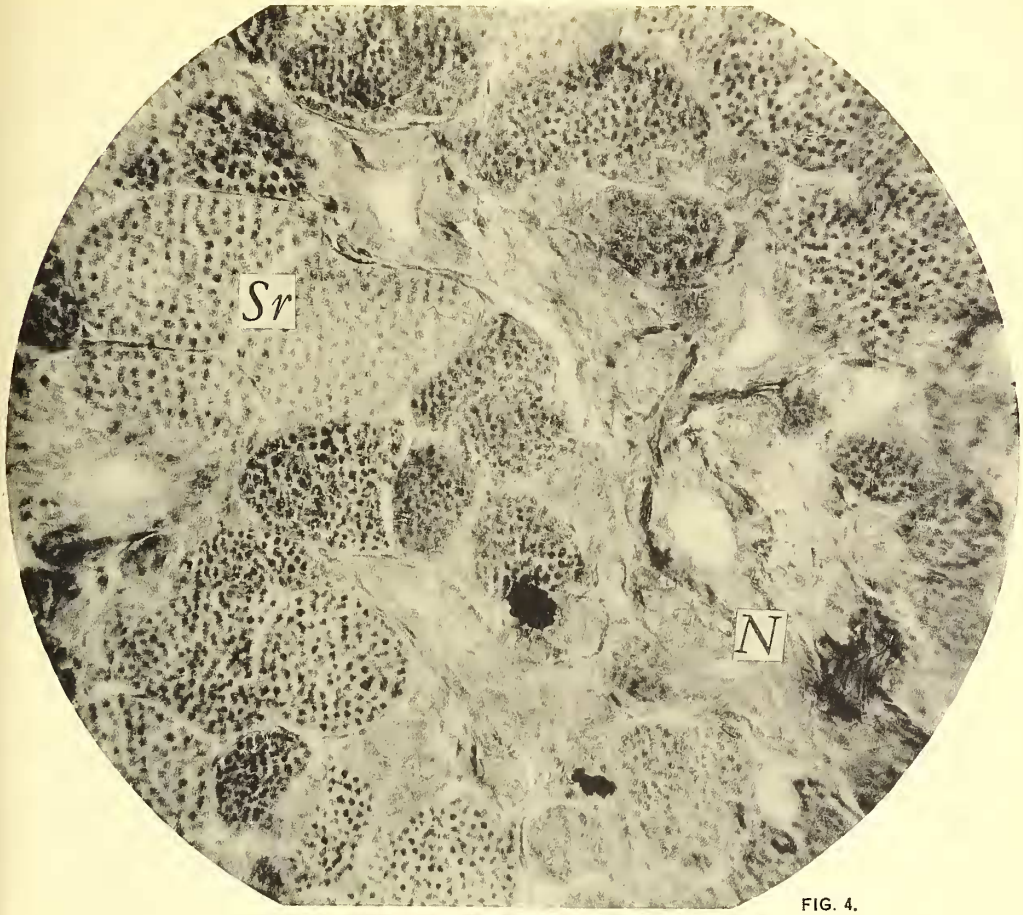

FIG. 4.

FIG. 5.

EFFECTS OF SEX HORMONES ON THE DEVELOPMENT OF THE PLATYFISH, PLATYPOECILUS MACULATUS.

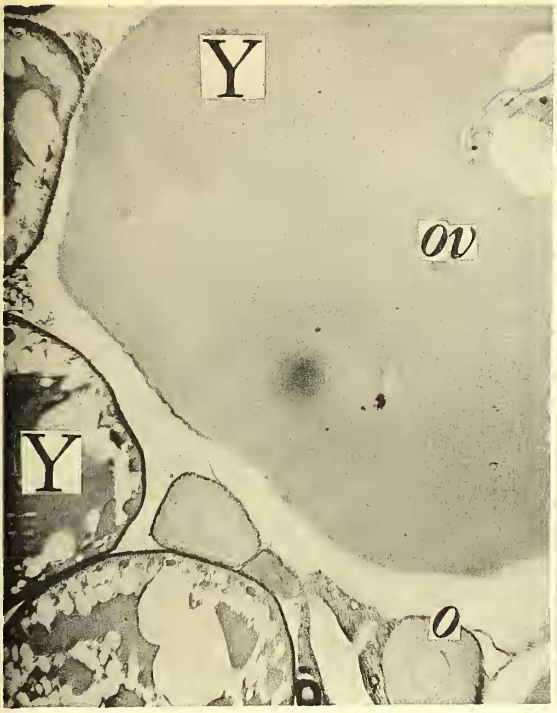

FIG. 6.

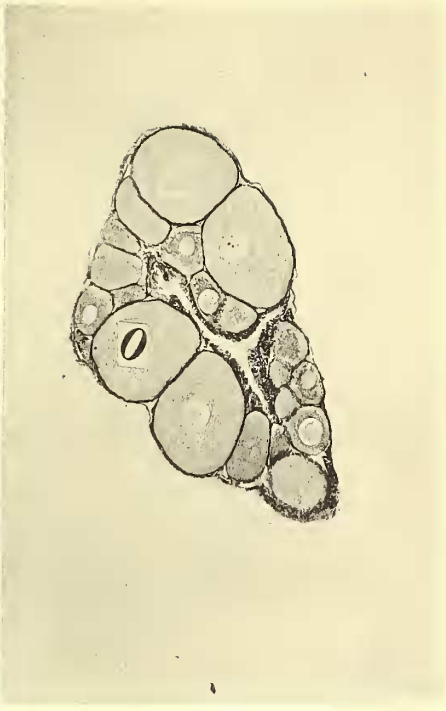

FIG. 7.

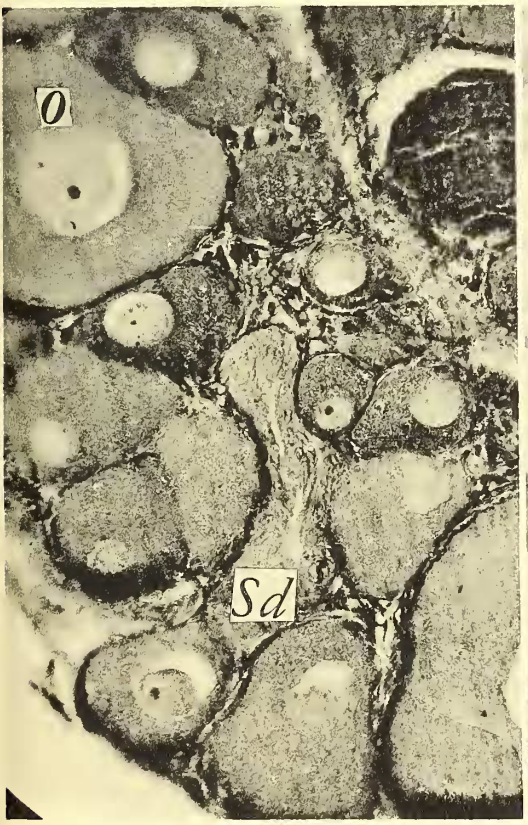

FIG. 8.

FIG. 9.

EFFECTS OF SEX HORMONES ON THE DEVELOPMENT OF THE PLATYFISH, PLATYPOECILUS MACULATUS.

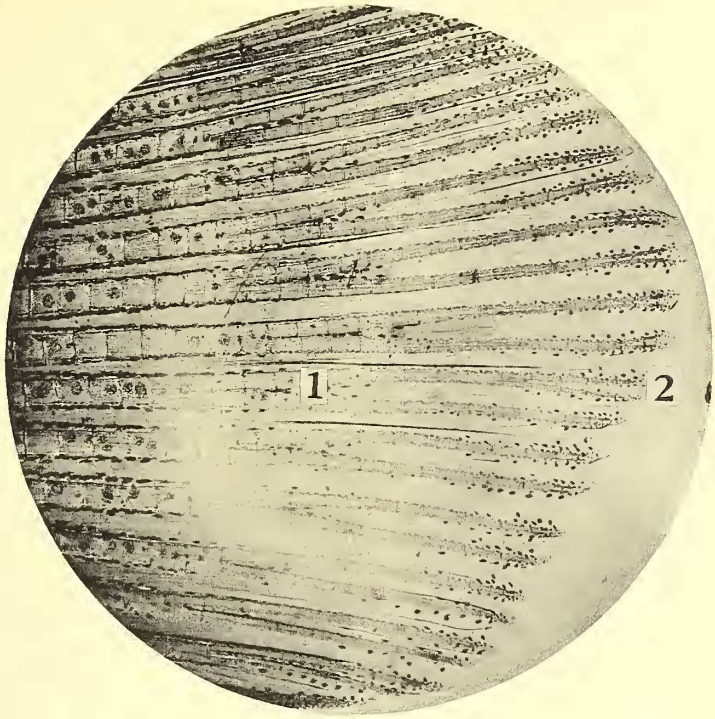

FIG. 10.

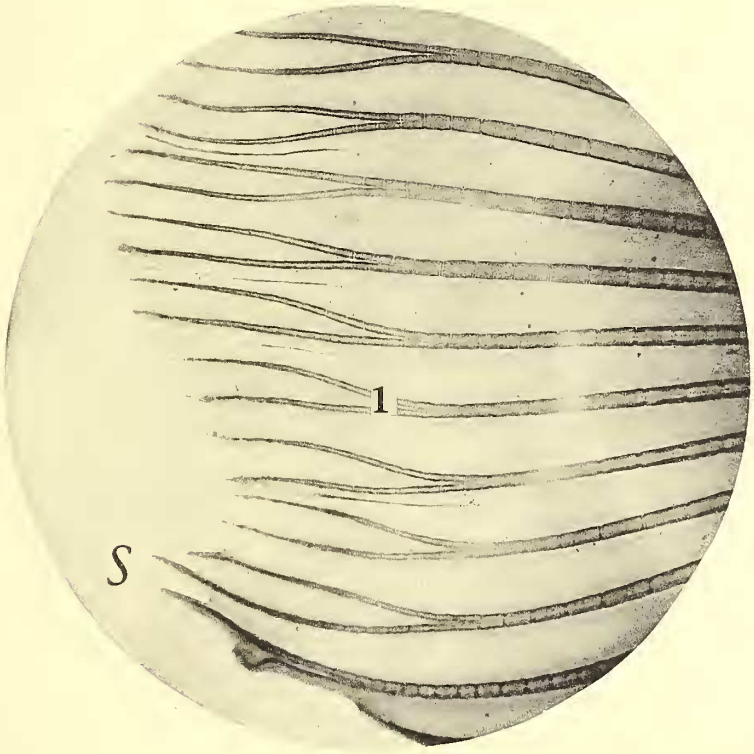

FIG. 11.

EFFECTS OF SEX HORMONES ON THE DEVELOPMENT OF THE PLATYFISH, PLATYPOECILUS MACULATUS.

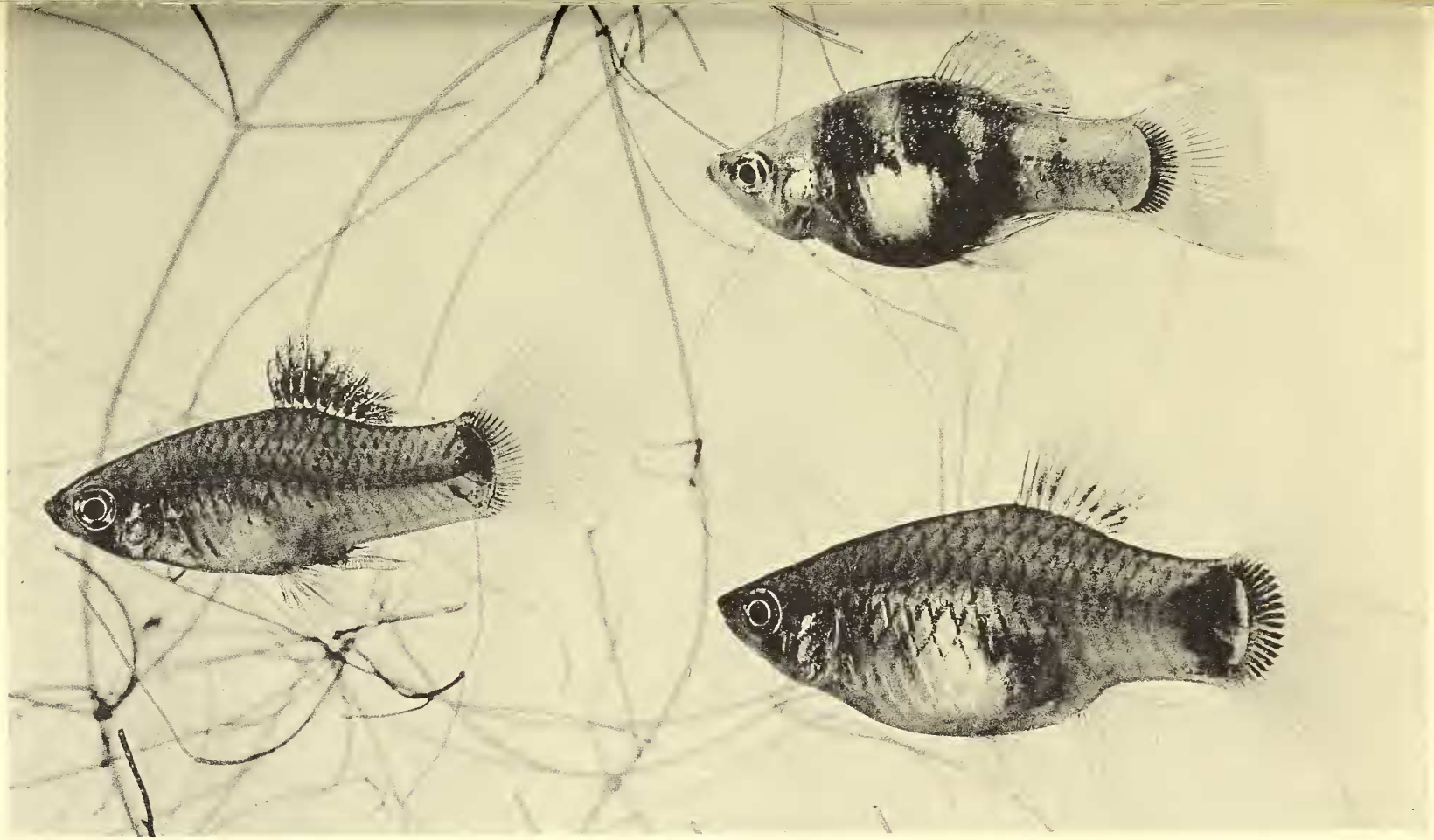

FIG. 12.

EFFECTS OF SEX HORMONES ON THE DEVELOPMENT OF THE PLATYFISH, PLATYPOECILUS MACULATUS.